

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :  
Ulrich Klar et al. : Group Art Unit:1614  
Serial No.: 09/485,292 : Examiner: Binta Robinson  
Filed: 3 May 2000 :

For: NEW EPOTHIOLONE DERIVATIVES, PROCESS FOR THEIR PRODUCTION,  
AND THEIR PHARMACEUTICAL USE

132 DECLARATION

Sir:

I, Ulrich Klar, being duly warned, declare that:

I am an inventor identified in the above-captioned application and am familiar with the invention described therein and with the grounds alleged for rejection made against the claims of the application in the Office Action mailed 4 March 2004.

The following experiments were conducted by me or under my supervision to show the advantage of replacement of the 6-methyl group in the natural compounds Epothilone B and Epothilone D by an ethyl or other higher alkyl substituent. The data unexpectedly demonstrate that the compounds with ethyl or other higher alkyl substituents have significantly better activity (i.e., lower  $IC_{50}$ ) in more tumor cell lines and better sensitivity to the multi-drug-resistant cell line NCI/ADR compared to MCF-7 than the corresponding compounds with methyl substitution.

The tumor cell lines used for the in vitro assays are of human origin. It is well accepted in the scientific community that the inhibition of tumor cell proliferation especially of different tumor cell lines of one tumor type (e.g. breast, lung, ovary etc.) are an indication that the compound may be useful in the treatment of this type of cancer also in vivo. Because not all of these tumor cell lines grow in vivo and due to our animal protecting laws, in vivo experiments can be performed only for a very limited number of compounds which are selected upon their in vitro profile.

In the following overview the unexpected beneficial effects observed by replacing the 6(10)-methyl group present in all naturally occurring epothilones by an alkyl-group is demonstrated.

The following data would demonstrate to the normally skilled researcher in this technology that the ethyl and higher alkyl compounds have significantly advantageous properties compared to the corresponding methyl compounds.

#### 1. Effect on activity

Compared are the IC<sub>50</sub> values obtained for different human tumor cell lines of a 6(10)-alkyl compound (right columns) with its corresponding 6(10)-methyl reference compound (middle columns). To demonstrate the broad usefulness of these unexpected findings, different types of epothilones are listed in Tables 1 to 4 as examples. Beside the naturally occurring epothilone B (Table 1) and epothilone D (Table 2) also synthetic analogs bearing several structural modifications at different regions of the molecule were investigated (Tables 3 to 4). The data unexpectedly demonstrate that the replacement of the 6(10)-methyl group by an alkyl group in different types of epothilones enhances the antiproliferative activity (lower IC<sub>50</sub> values).

Table 1: Replacement of the 6-methyl group in the natural compound Epothilone B by an ethyl group enhances the activity.

Table 1	 Epothilone B (Ref. 1)	
MCF-7	0.59 nM	< 0.24 nM
NCI/ADR	3.5 nM	0.43 nM
MaTu	0.46 nM	< 0.24 nM
MaTu/ADR	1.2 nM	< 0.19 nM
A 431	0.43 nM	< 0.1 nM
H460	0.35 nM	< 0.1 nM

Table 2: Replacement of the 6-methyl group in the natural compound Epothilone D by an ethyl group enhances the activity.

Table 2	 Epothilone D (Ref. 1)	
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MCF-7	19 nM	4.4 nM
MaTu	13 nM	6.3 nM
MaTu/ADR	37 nM	5.8 nM

Table 3: Replacement of the 6-methyl group in the synthetic reference compound 3 by a hydroxy-butyl group enhances the activity.

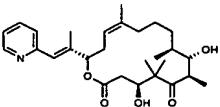
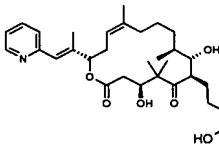
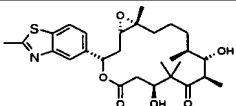
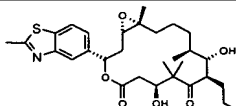
Table 3	 (Ref. 3)	
MCF-7	5.6 nM	3.5 nM
MaTu	5.4 nM	2.3 nM

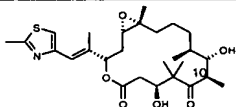
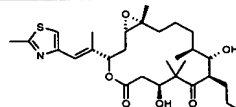
Table 4: Replacement of the 6-methyl group in the synthetic reference compound 4 by a propyl group enhances the activity.

Table 4	 (Ref. 4)	
MaTu	0.44 nM	0.2 nM
MaTu/ADR	0.81 nM	0.58 nM

## 2. Effect on overcoming multi-drug-resistance

Beside the overall activity it is desired not to lose activity against such human tumor cells which had already acquired marked resistancies. In Tables 5 to 10 the effect of replacing the 6(10)-methyl group by an alkyl group in different types of epothilones on the relative sensitivity to a human tumor cell lines overexpressing the multidrug resistance (MDR) phenotype is discussed. The relative sensitivity is defined as quotient of the IC<sub>50</sub>-values of a parent human tumor cell line (MCF7 or MaTu) and its corresponding MDR cell line (NCI/ADR or MaTu/ADR). This quotient is set to 100% for the reference compound bearing the 6(10)-methyl group. A value above 100% for the 6(10)-alkyl compound therefore indicates an improved sensitivity of the compound against the MDR cell line compared to its corresponding 6(10)-methyl reference compound.

Table 5: Replacement of the 6-methyl group in the natural compound Epothilone B by a propyl group enhances the relative sensitivity by 353%.

Table 5	 Epothilone B (Ref. 1)	
MCF-7	0.59 nM	3.4 nM

NCI/ADR	3.5 nM	4.3 nM
Ratio MCF7:NCI/ADR	0.17 (100%)	0.77 (453%)

Table 6: Replacement of the 6-methyl group in the natural compound Epothilone D by a propyl group enhances the relative sensitivity by 32% and 114%, respectively.

Table 6	Epothilone D (Ref. 2)	
MCF-7	19 nM	38 nM
NCI/ADR	50 nM	76 nM
Ratio MCF7:NCI/ADR	0.38 (100%)	0.5 (132%)
MaTu	13 nM	36 nM
MaTu/ADR	37 nM	48 nM
Ratio MaTu:MaTu/ADR	0.35 (100%)	0.75 (214%)

Table 7: Replacement of the 6-methyl group in the synthetic reference compound 7 by an ethyl group enhances the relative sensitivity by 208% and 186%, respectively.

Table 7	(Ref. 7)	
MCF-7	0.47 nM	1.6 nM
NCI/ADR	3.6 nM	4 nM
Ratio MCF7:NCI/ADR	0.13 (100%)	0.4 (308%)
MaTu	0.46 nM	1.2 nM
MaTu/ADR	1.3 nM	1.2 nM
Ratio MaTu:MaTu/ADR	0.35 (100%)	1.0 (286%)

Table 8: Replacement of the 6-methyl group in the synthetic reference compound 3 by an ethyl or propyl group enhances the relative sensitivity by 147% or 68%, respectively.

Table 8	(Ref. 3)		
MCF-7	5.6 nM	23.5 nM	26 nM
NCI/ADR	29 nM	50 nM	81 nM
Ratio MCF7:NCI/ADR	0.19 (100%)	0.47 (247%)	0.32 (168%)

Table 9: Replacement of the 6-methyl group in the synthetic reference compound 8 by an ethyl group enhances the relative sensitivity by 203% and 153%, respectively.

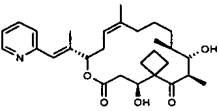
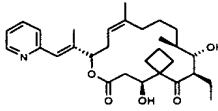
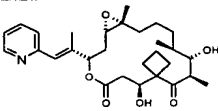
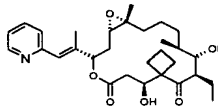
Table 9	 (Ref. 8)	
MCF-7	22 nM	33 nM
NCI/ADR	56 nM	28 nM
Ratio MCF7:NCI/ADR	0.39 (100%)	1.18 (303%)
MaTu	8.8 nM	31 nM
MaTu/ADR	29 nM	41 nM
Ratio MaTu:MaTu/ADR	0.30 (100%)	0.76 (253%)

Table 10: Replacement of the 6-methyl group in the synthetic reference compound 9 by an ethyl group enhances the relative sensitivity by 1592%.

Table 10	 (Ref. 9)	
MaTu	0.49 nM	1.1 nM
MaTu/ADR	4.1 nM	0.54 nM
Ratio MaTu:MaTu/ADR	0.12 (100%)	2.03 (1692%)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

 16.08.2004  
 Ulrich Klar Date